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V.A.C.™ instillation: *in vitro* model. Part 2

V.A.C.™ Instillation: ein *in vitro* Modell. Teil 2

Ludwig Labler* and Otmar Trentz

Division of Trauma Surgery, Department of Surgery, University Hospital Zurich, Zurich, Switzerland

Abstract

The behavior of a liquid in foam in the course of the V.A.C.™ instillation was investigated in an *in vitro* model by visualization using an aqueous color solution and by a quantitative determination of changing concentration of Ringerlactate solution.

Keywords: infected wound; V.A.C.; V.A.C. instillation; vacuum assisted closure.

Zusammenfassung

Das Verhalten einer Flüssigkeit im Schaumstoff während der V.A.C.™ Instillation wurde in einem *in vitro* Modell untersucht, visuell mit einer wässrigen Farbstofflösung und durch quantitative Bestimmung der sich ändernden Konzentration der Ringerlactat-Lösung.

Schlüsselwörter: V.A.C.; V.A.C. Instillation; Vakuumversiegelung; Wundinfektion.

Introduction

In Part 1 of our study [2], we presented an *in vitro* V.A.C.™ instillation model and investigated the instillation volume in dependence of time interval as well as the reproducibility of the system in general. As each instillation step leaves a part of the fluid, the residual volume, behind in the foam, the foam content is not completely removed and cannot be replaced by the next fluid portion in a single step [1, 2]. Instead, a supposedly rather complicated multi-step process has to be anticipated. In this study we used the above model and investigated the manner of fluid movements in the foam during instillation and V.A.C.™ phases by observation of a colored solution and by quantitative estimation of Cl^- concentrations of Ringerlactate solution in the course of a stepwise dilution with water.

*Corresponding author: Ludwig Labler MD,
Division of Trauma Surgery, University Hospital Zurich,
Rämistrasse 100, 8091 Zurich, Switzerland
Tel.: +41 44 255 11 11
Fax: +41 44 255 44 06
E-mail: ludwig.labler@usz.ch

Materials and methods

V.A.C.™ instillation *in vitro* model

All experiments in this study were performed with the model described in Part 1 [2] using a medium size foam (V.A.C.™ Medium Dressing Assembly, 18×12.4×3.3 cm, Kinetic Concepts Inc., San Antonio, TX, USA. cf. Part 1, figure 1a).

Fluid movement in the foam during instillation

The model as under 1. Non-glare glass sheet was used as foam support this time. The iv bottle was filled with a color solution (COLODUR Aqua-Buntlack Seidenmatt, weiss, J.W. Ostendorf, Zug, Switzerland, 50 ml were made up to 2000 ml with distilled water). The soft filter in the drip chamber of the infusion set was perforated to enable the colloid color solution to pass. The experiments were carried out with the foam on the glass sheet either in a horizontal or in a vertical position. When in vertical position, the two TRAC™ PADs were put on the foam either horizontally side by side, or vertically one upon another. The infusion tubing was then connected with the upper or with the lower PAD (center-to-center distance of the PADs was always 6 cm). Each experiment was performed with new foam. The infusion sets with perforated filters were used repeatedly. The foam was evacuated, the infusion tubing clamp opened and about 100 ml of the color solution (iv bottle scale) were let into the foam (instillation). The infusion tubing clamp was closed and a picture of the foam was taken through the glass sheet. The container clamp was then opened and the foam content transferred to the container (V.A.C.™ phase). The process was repeated until the container tubing was free of bubbles. Such a "stabilized" system was used for further experiments.

Replacement of fluids in the foam (simulation of subsequent instillations)

Color solution replaced by distilled water The iv bottle of the above described "stabilized" system was replaced by one filled with distilled water and equipped with a new infusion set (with intact filter). Instillations of 100 ml were followed by V.A.C.™ phases as long as most of the color was replaced by water. Pictures were taken after each instillation.

Ringerlactate solution replaced by distilled water A "stabilized" system in horizontal position as under 1, iv bottle filled with Ringerlactate solution (Ringerlactate B. Braun, B. Braun Medical AG, Emmenbrücke, Switzerland). The iv bottle and the infusion set were disconnected and replaced by an iv bottle filled with distilled

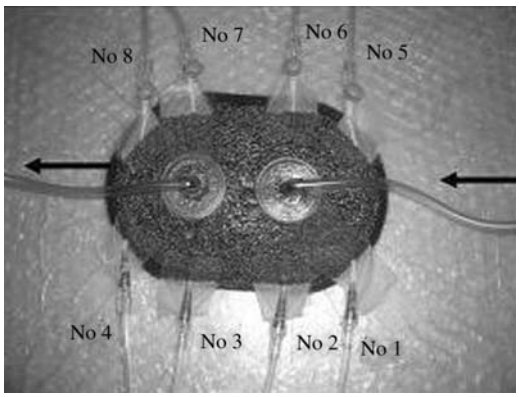

Figure 1 V.A.C.™ instillation model with catheters.
Catheters 1–4 are positioned 0.5 cm above the foam bottom, catheters 5–8 0.5 cm below the foam top.

water. The infusion set was replaced by a new one. The tare weight of both the bottle and the container was estimated. The instillation intervals were varied (range 20–90 s) under constant hold periods (60 s). Each instillation interval included ten instillations. The weight of inlets and outlets was estimated and expressed in milliliters. The infusion set and the screw cup were replaced by new ones when the iv bottle was refilled. The outlets were collected and finally the sponge squeezed to get a sample of the residual volume. The Cl^- concentration in the outlets and in the residual volume was estimated volumetrically in 5 ml samples. A theoretical course of the dilution was calculated using the mean outlet volume (ml), the mean residual volume of 78 ml determined for the medium size foam (cf. Part 1) and by means of the formula

$$c_{n+1} = \text{residual volume}/(\text{residual volume} + \text{outlet}) \times c_n (c = \text{concentration in \% Ringerlactate; starting } c_1 = 100\%) \quad (1)$$

Fluid movement in different foam locations The system as described above, except that eight iv catheters (Insyte 14 GA, Vialon, Spain or Delta Ven 2, Delta Med s.r.l., Viadana, Italy) were introduced into dry foam. Four catheters were positioned 0.5 cm above the foam bottom (nos 1–4) and the remaining ones (nos 5–8) 0.5 cm below its top (Figure 1). The points of the needles of each group of four catheters were side by side on the same level. The catheters were sealed tightly with an adhesive drape (OpSite Flexigrid, Smith + Nephew Medical Limited, Hull, England). Each catheter was equipped with a 20 cm tubing (G-Set, Galpro srl, Alessandria, Italy) and a stopcock (Discofix C, B Braun, Melsungen AG, Melsungen, Germany). Finally, the system was stabilized by instillation with Ringerlactate solution as described above using approximately 100 ml inlets. The bottle and infusion set were disconnected and substituted by an iv bottle filled with distilled water. The aqueous inlets of 90 s were followed by a V.A.C.™ phase. After each instillation, samples were taken from the eight catheters by a 10 ml syringe. At first, 4 ml were withdrawn and discarded. A 5 ml sample was then collected for analysis. The Cl^- concentration was estimated volumetrically in 3 ml samples.

Volumetric Cl^- determination in Ringerlactate solution

The sample was diluted with distilled water. The Cl^- content, declared as 112.2 mmol/l in Ringerlactate B. Braun, was determined according to Mohr [3] by titration with N/20 $AgNO_3$ and sodium chromate solution as indicator. The estimated Cl^- concentration was expressed in % of Ringerlactate concentration (c = 100%).

Results

The mode of filling the foam with the color solution is shown in Figures 2 and 3. In horizontal position, the bottom area of the foam is filled with the fluid at once, leaving the upper part empty (Figure 2a–c, top and lateral view and view from below). Next instillations successively filled the upper room (Figure 2d–g). Figure 3 shows the foam in vertical position and different TRAC™ PADs arrangements and its stepwise filling with the color solution.

The manner of the fluid movement in the foam in the course of subsequent instillations was the next object of our study. The replacement of the foam content, removed during the V.A.C.™ phase, by another fluid portion (both being of the same composition in reality) was simulated in our model by two fluids distinguishable either by their physical or chemical nature. A “stabilized” system, i.e., a system where the air was almost completely removed from the foam, was used in all experiments. A replace-

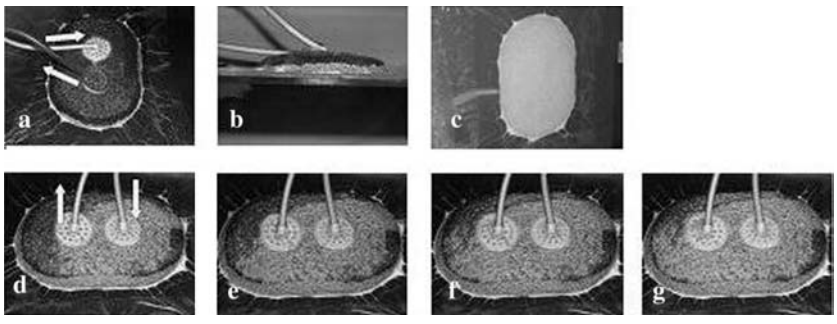

Figure 2 Foam in horizontal position.
Instillation with color solution. a–c: 1st Instillation, top and lateral view, view from below; d–g: 2nd–5th instillation, top view.

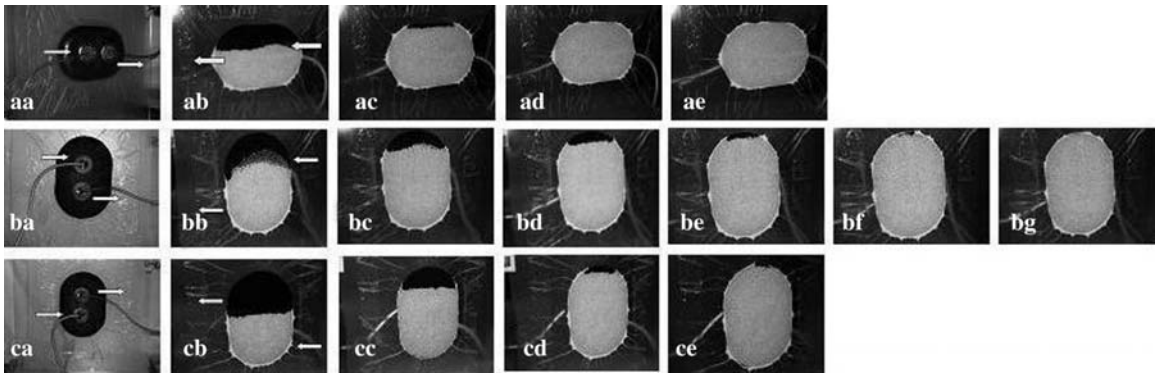

Figure 3 Foam in vertical position.
Instillation with a color solution. a: TRAC™ PADs horizontal. aa: front view; ab–ae: 1st–4th instillation, view from behind. b: TRAC™ PADs vertical, inlet above. ba: front view; bb–bg: 1st–6th instillation, view from behind. c: TRAC™ PADs vertical, inlet below. ca: front view; cb–ce: 1st–4th instillation, view from behind.

ment of a color solution by distilled water was chosen for a visual observation of the dilution process. The results of experiments carried out with foam both in horizontal and vertical position together with different TRAC™ PAD arrangements are illustrated in Figures 4 and 5. The dark coloration shows the distilled water portions delivered during instillations.

As the Cl^- concentration in Ringerlactate solution is easily estimated volumetrically [3], we chose a replacement of the former by distilled water for quantitative determination of its stepwise dilution in the outlets. As well as in the experiments described above, a "stabilized" system was used, filled with a Ringerlactate solution this time. The instillation phase time interval in particular measurements was 20, 40, 60 and 90 s. The data of the decreasing Cl^- concentrations obtained from the outlets are summarized in Table 1. A graphical comparison of experimental and calculated dilution is shown in Figure 6. The foams before having been discarded were squeezed to get a sample of the residual volume which was analyzed as well (Table 1 and Figure 7).

The Cl^- concentrations in different foam locations were determined in a device (Figure 1) which allowed collecting analytical samples directly from the foam after each instillation. Analytical data obtained are summarized in Table 2. A schematic cross section of the foam after seven instillations (Figure 8) provides a view of the concentration pattern in the foam. The Cl^- concentrations in the scheme are indicated both by numerals and by color gradation (100% Ringer solution = black, pure distilled water = colorless).

Discussion

The V.A.C.™ instillation, the recent modification of the negative pressure therapy, has proven to be a useful technique in the clinical practice for the treatment of infected and problematic wounds. Notwithstanding, what happens in the foam in general and the manner in which the fluid behaves in the course of the instillation phases has remained prevailingly unknown.

We were interested in the first place in the foam filling process and in the behavior of the fluid during subsequent instillations. Before the first instillation, a certain air volume is still present in the evacuated dry and collapsed

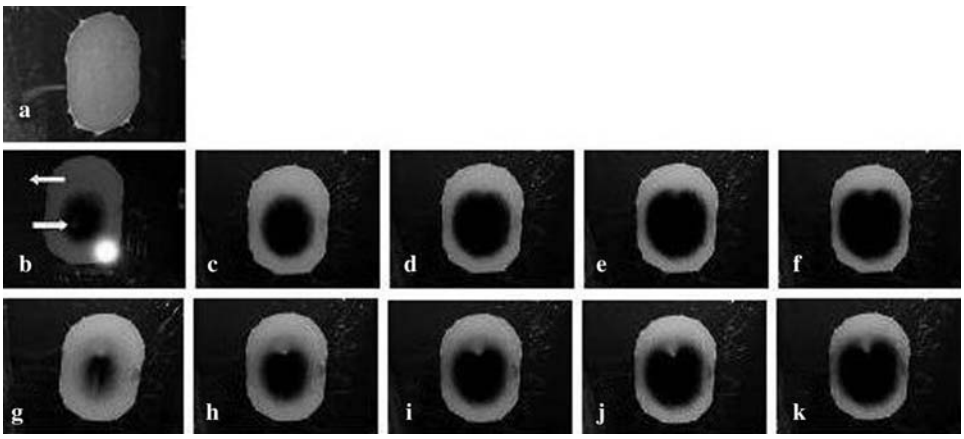

Figure 4 Foam in horizontal position.
Replacement of color solution by distilled water. a: Initial stage, stabilized system, view from below. b–f: 1st–5th instillation, view from below. g–k: 1st–5th V.A.C.™ phase, view from below.

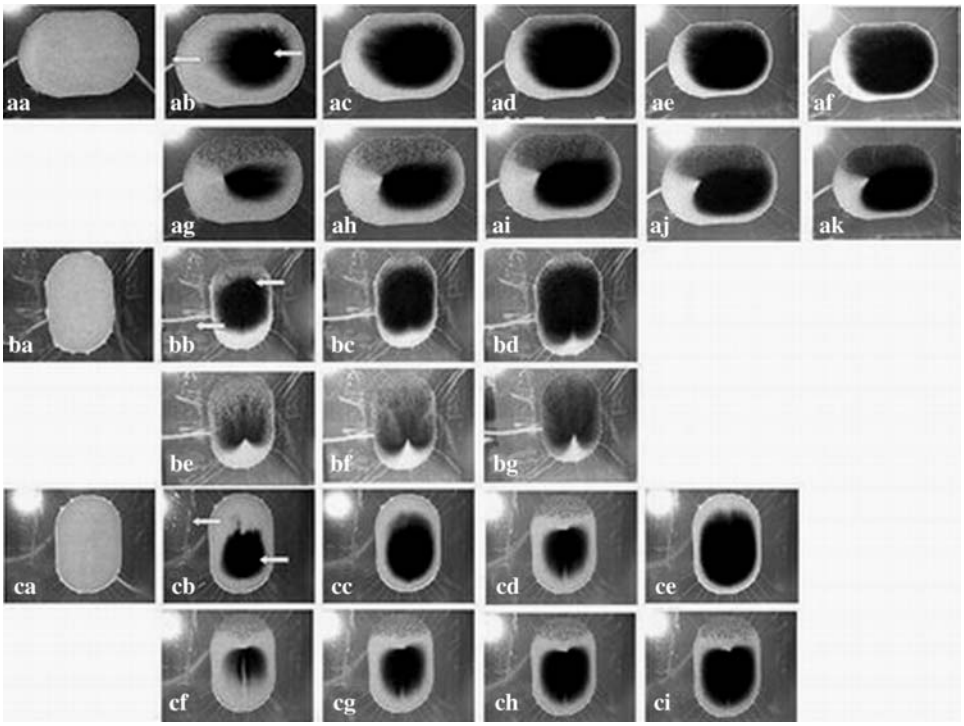

Figure 5 Foam in vertical position.
Replacement of color solution by distilled water. a: TRAC™ PADs horizontal. aa: Initial stage, stabilized system, view from behind; ab–af: 1st–5th instillation, view from behind; ag–ak: 1st–5th V.A.C.™ phase, view from behind. b: TRAC™ PADs vertical, inlet above. ba: Initial stage, stabilized system, view from behind; bb–bd: 1st–3rd instillation, view from behind; be–bg: 1st–3rd V.A.C.™ phase, view from behind. c: TRAC™ PADs vertical, inlet below. ca: Initial stage, stabilized system, view from behind; cb–ce: 1st–4th instillation, view from behind; cf–ci: 1st–4th V.A.C.™ phase, view from behind.

foam. To fill the foam more or less completely with the fluid, several instillations and V.A.C.™ phases are needed before the bubbles are removed from the container tubing [2]. To visualize the process of the foam filling a diluted aqueous white color solution was used. The fluid completely fills the whole bottom area of the horizontally positioned foam already during its first delivery into the collapsed dry foam (Figure 2b,c). The upper part of the foam remains empty (Figure 2a) and fills stepwise in the course of the following instillations (Figure 2d–g). Nevertheless, even after five liquid deliveries, small, disconnected empty areas are still visible by darker coloration (Figure 2g). The foam in clinical practice serves as a drug-release system and only the parts of the foam contacting the wound, i.e., the bottom surface and the sides, are pertinent for the physiological action. In view of Figure 2, the system seems to be in full action already after the first solution delivery at least as concerns the bottom area. The extent of participation of the sides on the drug release in only partly filled foam is difficult to define. The literature recommends fixing the V.A.C.™ instillation system [4] always in a horizontal position. The customary V.A.C.™ therapy, however, has been applied to cover wounds in all possible body locations. It was of interest how, if at all, the system worked in a vertical position. Figure 3 shows the results obtained with all three possible positions of the PADs. The foams were filled upwards as anticipated, regardless of the position of the delivery tubing, and an entirely fully active area was not achieved before several instillations, their number depending on the PADs' arrangement. Anyhow, the vertical arrangement, as we see it, when repeated preliminary instillations were accepted, might be worth trying in the practice.

The above experiments merely show how the foam fills irrespective of the liquid behavior in the course of subsequent instillations. The process proceeds in fact between a new delivery and the remainder in the foam, the residual volume. The residual volume, remaining in the evacuated foam and amounting to 78 ml on average for the foam of medium size [2], is in no way negligible. Consequently, the instillation is not a single and simple replacement of solutions as assumed [1] but a supposedly complicated multi-step dilution process. In our model experiment, we had to distinguish between the fluid in the foam and the replacing liquid. For this purpose and to eliminate the irregularities during the first few instillations, we used a "stabilized" system filled with the colored solution as described above and the instillations that followed were then performed with distilled water. The experiment was started with the foam in horizontal position (Figure 4). The visualization of the process is naturally limited to the bottom area only. There is always a dark water area, shifted towards the delivery PAD, and its white surrounding of the color solution which does not disappear even after several instillations. Both areas are separated by a narrow gray ring of a mixture of both phases indicating a very limited diffusion of the liquid phases in the foam. It should not be forgotten that in reality the foam bottom is opened towards the wound

Table 1 Replacement of Ringerlactate solution by distilled water (inlet variable, hold[1)] constant) (experimental and calculated[2)] course).

	Inlet/outlet no	Inlet 20 s					Inlet 40 s					Inlet 60 s					Inlet 90 s				
		Inlet (ml)	Outlet (ml)	N/20 $AgNO_3$ (ml)	Ringer solution Cl^- conc (%)	Calculated	Inlet (ml)	Outlet (ml)	N/20 $AgNO_3$ (ml)	Ringer solution Cl^- conc (%)	Calculated	Inlet (ml)	Outlet (ml)	N/20 $AgNO_3$ (ml)	Ringer solution Cl^- conc (%)	Calculated	Inlet (ml)	Outlet (ml)	N/20 $AgNO_3$ (ml)	Ringer solution Cl^- conc (%)	Calculated
Ringer water			40	11.1	100.0			64	11.4	100.0			90	11.75	100.0			90	11.60	100.0	
	1	40	42	9.4	84.7	66.1	66	64	6.6	57.9	55.3	90	90	5.60	47.7	47.0	114	114	5.00	43.1	40.8
	2	40	40	4.2	37.8	43.7	66	66	2.6	22.8	30.6	90	88	2.05	17.4	22.1	116	116	1.90	16.4	16.7
	3	42	42	2.9	26.1	28.9	64	64	1.7	14.9	16.9	86	88	1.20	10.2	10.4	116	116	1.00	8.6	6.8
	4	40	42	2.1	18.9	19.1	64	64	1.2	10.5	9.4	86	88	0.80	6.8	4.9	114	114	0.70	6.0	2.8
	5	44	40	1.6	14.4	12.6	64	62	0.9	7.9	5.2	88	88	0.60	5.1	2.3	112	112	0.45	3.9	1.1
	6	38	40	1.3	11.7	8.3	64	62	0.7	6.1	2.9	90	88	0.40	3.4	1.1	114	114	0.30	2.6	0.5
	7	42	38	1.0	9.0	5.5	62	62	0.5	4.4	1.6	88	88	0.25	2.1	0.5	112	114	0.20	1.7	0.2
	8	40	40	0.9	8.1	3.6	64	62	0.4	3.5	0.9	88	88	0.20	1.7	0.2	110	112	0.20	1.7	0.1
	9	40	38	0.8	7.2	2.4	62	62	0.4	3.5	0.5	88	88	0.15	1.3	0.1	114	112	0.10	0.9	0.0
	10	40	42	0.6	5.4	1.6	60	62	0.3	2.6	0.3	90	88	0.15	1.3	0.1	108	110	0.10	0.9	0.0
Residual volume				2.0	18.0	1.6			1.1	9.6	0.3			0.70	6.0	0.1			0.45	3.9	0.0

[1)] Hold 60 s, samples for analysis: 5 ml. [2)] Calculated values: c_{n+1} = residual volume/(residual volume + outlet) * c_n (c = concentration in %; starting c_1 = 100%; (20 s average outlet = 40 ml; 40 s average outlet = 63 ml; 60 s average outlet = 88 ml; 90 s average outlet = 113 ml; average residual volume = 78 ml [2]).

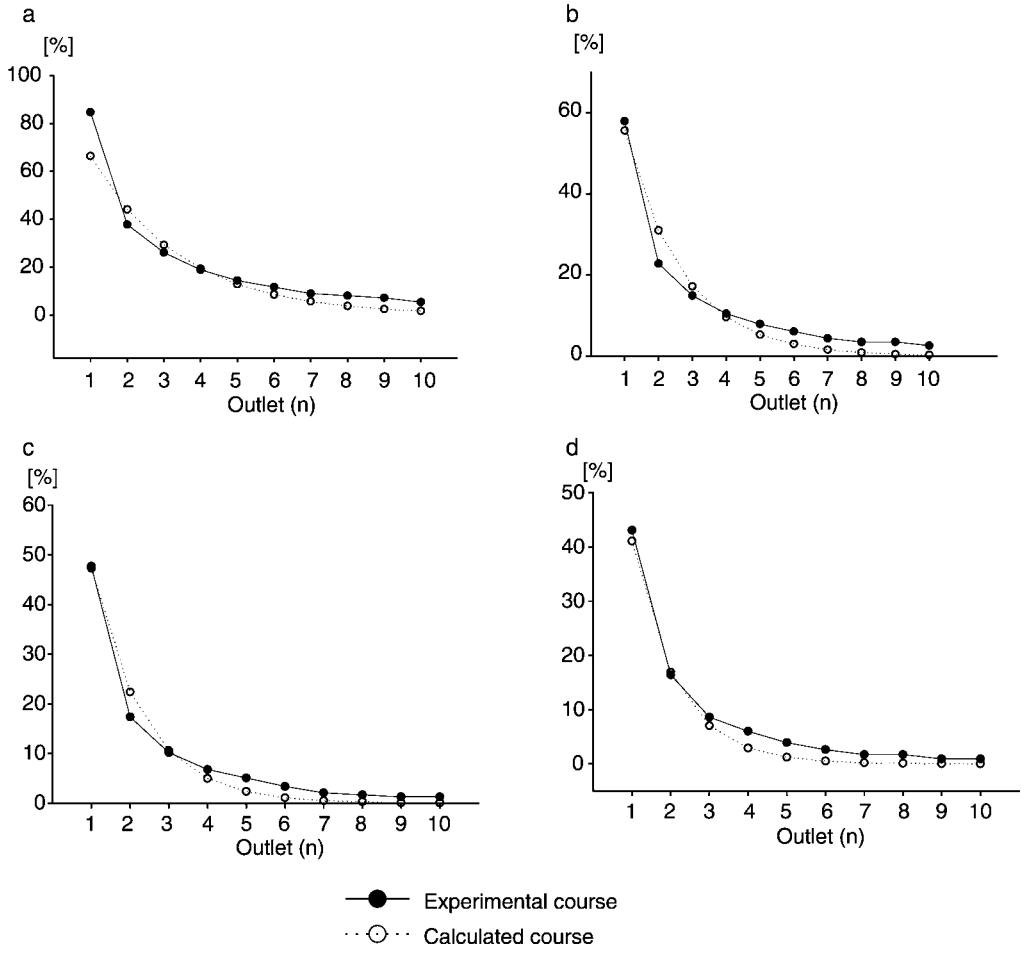

Figure 6 Replacement of Ringerlactate solution by distilled water: experimental and calculated course.
Foam horizontal, instillation phase variable, stabilized system, initial stage: Ringerlactate solution = 100%. a: Instillation phase 20 s. b: Instillation phase 40 s. c: Instillation phase 60 s. d: Instillation phase 90 s.

surface, which is not realizable in the model. Whereas the model system comes to a standstill after removal of the foam content during the V.A.C.™ phase, in the wound an undefined quantity of the wound secret is sucked in and removed and the fluid in the foam is in constant movement. The residual volume, which comes in contact with the next instillation, then consists supposedly mostly of the wound secret. Thus, the photographs of the V.A.C.™ phases, shown in Figure 4g–k, should be interpreted carefully. The instillation process in vertically positioned foams mostly proceeds in a similar manner (Figure 5). The pictures of the V.A.C.™ phases contain, besides the dark water and white color area, larger gray areas in upper foam parts (Figure 5ag–ak, be–bg, cf–ci). We take them for empty pockets left behind after the fluid was shifted down by their own weight. In the corresponding pictures of the foam in horizontal position, this phenomenon is absent because it takes place supposedly in the upper part of the foam.

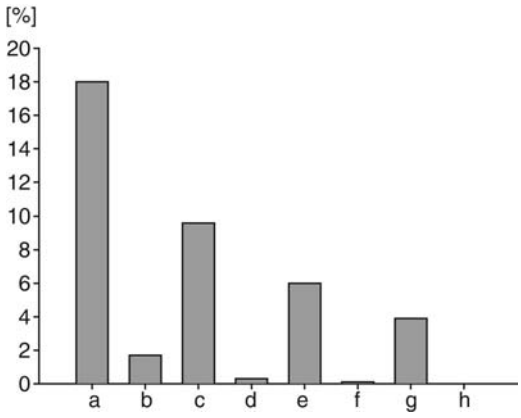

Figure 7 Replacement of Ringerlactate solution by distilled water: concentration in residual volume.
See Figure 6, % of Ringerlactate. Experimental: a: 20 s; c: 40 s; e: 60 s; g: 90 s. Calculated: b: 20 s; d: 40 s; f: 60 s; h: 90 s.

For a quantitative determination of the dilution process we used Ringerlactate solution and distilled water. As described above, the system in horizontal position was stabilized beforehand with a Ringerlactate solution. Ten instillations with distilled water were carried out in four different time intervals. In order to maintain standard conditions, a constant hold phase interval of 60 s was put in between instillation and V.A.C.™ phases. The decrease of the Cl^- concentration was estimated in the outlets and compared with the calculated values (Table 1 and Figure 6). The experimental course and the deviations from the calculated course are similar in all four measurements, indicating a limited diffusion of the liquid and its non-uniform distribution in the foam. This applies, above all, to the first outlet with a concentration in part markedly

higher than calculated. It seems that the remote foam content is pushed more or less undiluted forward into the container. The concentrations of the following outlets and their alternation towards the calculated values are less clear and we do not have a satisfactory answer for it. The same applies to the surprisingly high Cl^- concentrations, proportionally reversed to the instillation volume, of the residual volumina obtained from squeezed foams (Table 1 and Figure 7).

An approximate view of what goes on inside the foam gave the experiment shown in Figure 1. By means of eight catheters introduced into different parts of the foam, samples were collected after each instillation and their Cl^- concentrations estimated (Table 2). A schematic cross section of the foam (Figure 8) illustrates the irregular distribution of the concentrations. The top cross section (first inlet) shows the shift of higher concentrations in direction of the outlet PAD in agreement with the observation discussed above. Although the concentrations in the particular sectors of the foam differ substantially, their mean value agrees remarkably well with the calculated data (Table 2).

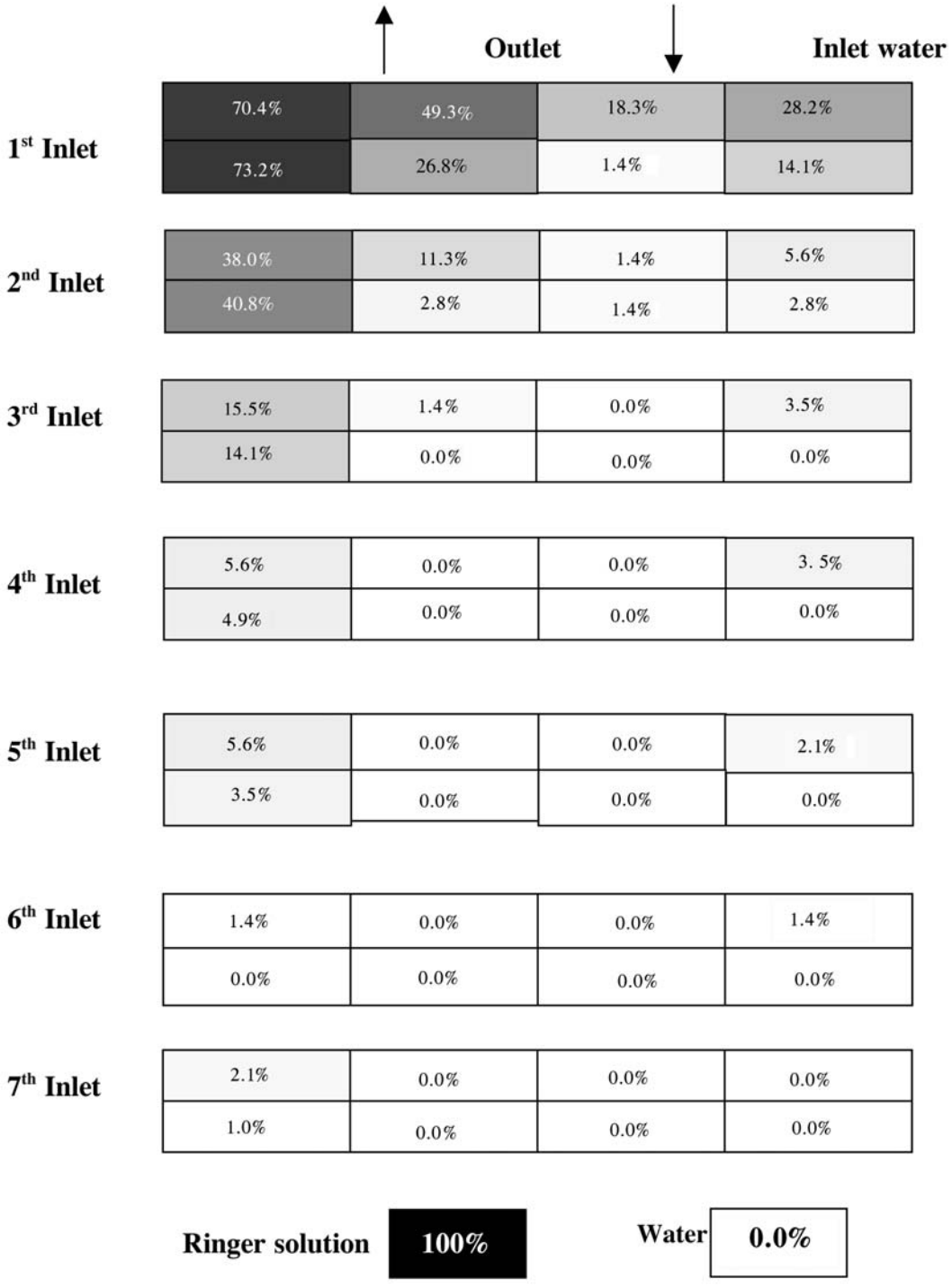

Figure 8 Replacement of Ringer solution by distilled water. Concentrations in foam locations. (Schematic longitudinal foam cross section.)

Table 2 Replacement of Ringerlactate solution by distilled water. Concentrations in different foam locations.

Outlet no	Inlet (ml water)	Catheter no and concentration (%)				Concentration of Ringerlactate solution in foam	
		8	7	6	5		
		4	3	2	1	Mean	Calculated[1]
1	140	70.4	49.3	18.3	28.2		
		73.2	26.8	1.4	14.1	35.2	35.8
2	139	38.0	11.3	1.4	5.6		
		40.8	2.8	1.4	2.8	13.0	12.9
3	138	15.5	1.4	0.0	3.5		
		14.1	0.0	0.0	0.0	4.3	4.7
4	130	5.6	0.0	0.0	3.5		
		4.9	0.0	0.0	0.0	1.8	1.8
5	124	5.6	0.0	0.0	2.1		
		3.5	0.0	0.0	0.0	1.4	0.7
6	124	1.4	0.0	0.0	1.4		
		0.0	0.0	0.0	0.0	0.4	0.3
7	126	2.1	0.0	0.0	0.0		
		1.0	0.0	0.0	0.0	0.4	0.1

Mean residual volume = 78 ml [2], samples for analysis: 3 ml. [1] Calculated concentration: $c_{n+1} = c_n * 78/(78 + \text{inlet})$.

In conclusion, as demonstrated in the model, V.A.C.™ instillation is a rather complicated process. An important additional circumstance which has to be taken into consideration is the residual volume probably consisting mostly of a wound secret which then dilutes and mixes in some way with the physiological solution in the foam during instillations. Because of its not negligible amount, a reasonable instillation volume is necessary. Instillation intervals carried out under conditions as described in our study should, in our opinion, amount to between 60 and 120 s. Shorter instillations were of little use being too small in relation to the residual volume. Instillations over two minutes are also not justified, because the foam produces a pressure on the covering sheet and simultaneously the liquid starts to get out of the foam and works its way under the transparent adhering sheet. Last but not least it seems that the technique might be of use also in other positions than the horizontal one.

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